IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Eduardo M. Lasalvia-Prisco

Serial No.: 10/607,358

Filing Date: June 26, 2003

For: A METHOD AND COMPOSITION TO ELICIT AN EFFECTIVE AUTOLOGOUS ANTITUMORAL IMMUNE RESPONSE IN A PATIENT

Confirmation No. 6124
Customer No. 04219
Group Art Unit 1643
Sang, Hong, Examiner

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

DECLARATION OF DR. EDUARDO M. LASALVIA-PRISCO

I, Dr. Eduardo M. Lasalvia-Prisco, declare as follows:

1. That I am the sole applicant and inventor of the new and inventive method and composition disclosed and claimed in the above-referenced U.S. Patent Application having Serial Number 10/607,358, entitled A METHOD AND COMPOSITION TO ELICIT AN EFFECTIVE AUTOLOGOUS ANTITUMORAL IMMUNE RESPONSE IN A PATIENT (hereinafter "the Application").

2. That I am a Medical Doctor and Oncologist, currently licensed in Uruguay, and that through my extensive clinical and academic practice, and authorship of medical research papers over a period of more than 30 years, I have gained a significant

level of expertise in the field of Oncology.

3. That I the Director of Research and Development at Pharmablood Inc., Miami, Florida; that I was a Professor at the Medical School of the University of Uruguay; that I am an Internist, Internal Medicine Degree, Post-Graduate School of the School of Medicine, University of Uruguay; that I am an Oncologist, Clinical Oncology Degree, Post-Graduate School of the School of Medicine, University of Uruguay; that I have been granted a fellowship in Cancerologie, University of Paris, Institute of Cancerologie et d'Immunogenetique; that I have been awarded Villejuif: Certificate of Improvement in Cancerologie; that I am a recipient of a Scholarship of Improvement of the Government of France; and I am an active member of the American Society of Clinical Oncology, the Society of Internal Medicine of Uruguay, the Society of Hematology of Uruguay, and the European Organization for the Research of Cancer.

4. That I have conducted research in oncology throughout my career to identify new treatments, develop products, define their rationale, design, coordinate, and evaluate clinical trials for oncological research involving teams in different countries; I have authored or co-authored the publications on the following topics, among other: Antiprogressive Immunotherapy Using an Autologous Hemoderivative; the Role of Tumor Associated Antigens and Regulatory Cells [CD4+CD25+] as Targets of the

Immune Response Elicited by an Anti-progressive Autologous Hemoderivative; Autologous Immunotherapy Optimized by indoleamine-2,3-dioxygenase (IDO)-Inhibitor as Immune-Tolerance Breaker; Extraction of an Inhibitor of DNA Synthesis From Human Peripheral Blood; and, Evidence for Lymphocytic Chalone.

5. That attached hereto as Exhibit A is a true and accurate copy of my Curriculum Vitae which includes a listing of Specialties and Post-Graduate Studies; Awards, Scholarships, Distinctions, Technical and Scientific Missions; Faculty Positions - School of Medicine; Professional Activity - Clinical Practice; Professional Activity - Pharmaceutical Industry; Activities in Medical Education and Health Policy Planning; Scientific Societies; Scientific Publications - Board Member; Scientific Publications - Invitation as Reviewer, as well as a list of some of the scientific publications which I authored or co-authored that are relevant to the field of oncology.

6. That the inventive method and composition disclosed and claimed in the Application is based upon my research in cancer immunotherapy, specifically, developing an immunogen or vaccine prepared from the patient's own blood to produce an autologous vaccine via a procedure not previously known in cancer immunotherapy or otherwise, and that through this research I discovered that subjecting a solution derived from the supernatant of a sedimented blood specimen to specific temperatures for specific periods of time produces an autologous

vaccine which may be effectively employed in active specific immunotherapy to produce desirable clinical effects.

7. That I am the principal co-author of an article entitled "Antitumoral Effect of a Vaccination Procedure with an Autologous Hemoderivative," which was published in Cancer Biology & Therapy in February 2003 (hereinafter "the CBT Article"), and which reports the results of initial clinical trials utilizing the inventive method and composition as disclosed and claimed in the Application.

8. That attached hereto as Exhibit B is a true and correct copy of the commentary entitled "A New Twist on Autologous Cancer Vaccines," by Leisha A. Emens, M.D., Ph.D., Professor of Oncology, The Sidney Kimmel Comprehensive Cancer Center, as published in Cancer Biology & Therapy in February 2003 (hereinafter "the Emens Commentary").

9. That upon information and belief, Dr. Leisha A. Emens is qualified as an Expert by John Hopkins University; and that Dr. Emens is the Principal Investigator for ongoing research directed towards vaccine therapy with or without cyclophosphamide and doxorubicin in women with stage IV breast cancer, sponsored by the National Cancer Institute.

10. That the Emens Commentary is an unsolicited and independent review and analysis of the results of initial clinical trials utilizing the method and composition in

accordance with the invention as disclosed and claimed in the Application, and as published in the CBT Article.

11. That the Emens Commentary, paragraph 1, with reference to the method and composition as disclosed and claimed in the Application and as published in the CBT Article, states that the "... approach is intriguing because it circumvents many of the practical obstacles to the development of effective vaccines for cancer therapy."

12. That the Emens Commentary, paragraph 4, states that "Lasalvia-Prisco and colleagues describe a novel vaccine formulation derived from the arterial blood of advanced solid tumor patients. They develop a procedure for manufacturing and partially characterizing an autologous hemoderivative."

13. That the Emens Commentary, paragraph 5, also with reference to the method and composition as disclosed and claimed in the Application and as published in the CBT Article, states that "... this vaccine platform in principle offers multiple advantages. First, the small quantity of arterial blood required is quickly and safely accessible by femoral arterial puncture. Second, the manufacturing process is relatively simple and cost-effective, requiring minimal manipulation and no direct chemical or genetic modification of the cellular component prior to processing. Finally, and most importantly, the patient himself is a renewable source of therapeutic

material. At the time of tumor recurrence, an updated vaccine product that accurately reflects that antigenic profile of the tumor at that point in time could be easily obtained and prepared."

14. That the Emens Commentary, paragraph 10, states that "[i]n summary, Lasalvia-Prisco and colleagues have described a novel cancer vaccine platform consisting of an autologous hemoderivative, with a suggestion of clinical response."

15. That the Emens Commentary, paragraph 10, once again, with reference to the method and composition as disclosed and claimed in the Application and as published in the CBT Article, states that "[c]learly, an active, individualized cancer vaccine that is cost-effective and simple to manufacture would be a welcome addition to the treatment armamentarium for metastatic solid tumors."

16. That the Emens Commentary is independent corroboration by a person having considerable skill in the art that the invention as disclosed and claimed in the Application is novel, unique, and intriguing, and "offers multiple advantages [relative to known techniques]," thereby evidencing that the invention as disclosed and claimed in the Application is not "obvious" to one of ordinary skill in the art, as cited in the Office Action as a basis to reject the claims which remain pending in the Application.

17. That the Emens Commentary is independent corroboration by a person having considerable skill in the art, evidencing that the invention as disclosed and claimed in the Application "would be a welcome addition to the treatment armamentarium for metastatic solid tumors," thereby evidencing that the invention as disclosed and claimed in the Application fulfills a long felt need in the art.

18. That I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: October 20, 2006

Dr. Eduardo M. Lasalvia-Prisco

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EDUCATION

- Doctor in Medicine 1968. Residency 1968-1970: Medical School, University of Uruguay (1961-1968)
- Degree in Biological Sciences, 1969: School of Sciences, University of Uruguay

SPECIALTIES AND POST-GRADUATE STUDIES 1975- 1985

- Internist, Internal Medicine Degree, Post-Graduate School of the School of Medicine, University of Uruguay
- Oncologist, Clinical Oncology Degree, Post-Graduate School of the School of Medicine, University of Uruguay
- Specialist in Clinical Laboratory, Biochemistry
- Cancerologie, University of Paris, Institute of Cancerologie et d'Immunogenetique. Villejuif: Certificate of Improvement in Cancerologie, 1970
- Hematology, University of Paris, Paul Brousse Hospital, Group Hopitalier Gustave Roussy. Certificate of Improvement in Hematology, 1976

AWARDS, SCHOLARSHIPS, DISTINCTIONS, TECHNICAL AND SCIENTIFIC MISSIONS

In Uruguay, the School of Medicine has two annual Awards, the Annual Scholarships that are granted to the 2 graduates with the most outstanding academic merits according to a special tribunal. The University of the Uruguay also grants every year among the graduates of all the University Schools that have obtained the Annual Scholarships, two awards denominated Artigas Scholarships, to those who gather the highest academic merits according to a special tribunal. Independently, several types of Scholarships for studies of Improvement are offered by different Governments to the university graduates who are selected by their educational and academic records.

- Annual Scholarship, School of Medicine, University of Uruguay, 1968
- Artigas Scholarship, University of Uruguay, 1968
- Scholarship of Improvement of the Government of France
- Scholarship as Invited Investigator of the Government of France

- Mission of Oncological Research Coordination. Birbeck College London
- Mission of Multicentric Coordination in the Oncological Research Program, Department of Tumoral Biology. Institute Karolinska. Stockholm. Sweden
- Scholarship of Improvement of the Government of Spain. Hematology Program. Farreras Valenti-Ciril Rozman Clinic, Clinical Hospital, Barcelona, Spain
- Title of Master of the Latin American Medicine. Argentina Medical Association, Argentina
- Mission of the Ministry of Health of Uruguay to the National Cancer Institute, USA
- Uruguayan delegate to the Pan-American Heath Organization Meeting
- Special guest to the Annual Meeting of the Executive Committee of the Pan-American Health Organization
- Special guest to the First International Congress of Italian Scientists in the World (Primo Convegno internazionale degli Scienziati italiani nel mondo (Roma, 10-12 marzo 2003), Government of Italy.
- Member of the Scientific Board of Journal of Clinical and Biological Research
- Member of the Scientific Board of Immunology and Immunotherapy
- Member of the Scientific Board of Cancer Chemotherapy and Pharmacology

FACULTY POSITIONS, SCHOOL OF MEDICINE (1963-1993)

The academic career in the School of Medicine of Uruguay, as well as in all the other Schools of the University of Uruguay, consists of 5 successive hierarchical levels (Grade 1 to 5). All the permanent positions are accessed by an open contest exam. Grade 5, Professor, is the highest position. The academic positions duties are: education of pre-degree and post-degree students, scientific research and, when appropriate, direct clinical practice in Public Hospitals. In Uruguay, there is only one School of Medicine, part of the University of Uruguay, which is a State University.

- Department Assistant 1 (Grade 1). Department of Biochemistry. School of Medicine, University of Uruguay
- Department Assistant 2 (Grade 2). Department of Biochemistry. School of Medicine, University of Uruguay
- Assistant Professor (Degree 3). Department of Biochemistry. School of Medicine, University of Uruguay
- Associate Professor (Degree 4). Department of Biochemistry. School of Medicine, University of Uruguay
- Professor and Chairman (Grade 5): 1977-1984 Department of Biochemistry. School of Medicine, University of Uruguay
- Resident, Department of Medicine (Grade 1)
- Department Assistant 2 (Grade 2). Department of Medicine. School of Medicine, University of the Uruguay
- Assistant Professor (Grade 3). Department of Medicine. School of Medicine, University of the Uruguay
- Associate Professor (Grade 4) Department of Medicine. School of Medicine, University of the Uruguay
- Professor and Chairman (Grade 5): 1984-1994 Department of Medicine. School of Medicine, University of the Uruguay

PROFESSIONAL ACTIVITY - CLINICAL PRACTICE (1963-2004)

The Health System in Uuguay has 2 sub-systems, one public and one private. The public health Subsystem offers coverage 50% of the population through a net of General Hospitals and Specialized Institutes. The private health Subsystem covers the rest of the population through pre-payment managed care organizations (equivalent to the Health Maintenance Organizations or HMOs).

- Director of the National Cancer Institute of Uruguay: 1977-2002 (Public Health System)
- Chairman of the Department of Medicine of the Uruguayan Medical Corporation, MUCAM (Private Health System).
- Chairman of the Oncology Department of the Uruguayan Medical Corporation, MUCAM (Private Health System).
- Chief of Staff, Clinical Oncology, of the Medical Center of the Medical Union of Uruguay, CASMU (Private Health System).
- Medical Director of Interdoctors, Medical Center (Private Health System).
- Chief of the Applied Biomedical Investigations Service. Ministry of Public Health of Uruguay (Public Health System)
- Laboratory Assistant, Ministry of Public Health of Uruguay (Public Health System)

PROFESSIONAL ACTIVITY – PHARMACEUTICAL INDUSTRY (1963-2004)

- Medical Director, in pharmaceutical/biotech companies. He has directed and executed clinical development programs (phase II, III) in Oncology. Expertise in providing scientific support and input to the evaluation of in-licensing opportunities, early development projects and phase IIIb/IV Clinical Development plans; develop Principal Opinion Makers and or Panel Discussants, within the scope of the Clinical Development Unit; support business development by identifying and evaluating business opportunities and by identifying and anticipating all industry market trends related to Oncology therapeutics.
- Design of new pharmaceutical products.
- All steps of product design, manufacturing, approval and marketing.

ACTIVITIES IN MEDICAL EDUCATION AND HEALTH POLICY PLANNING

- Organization and Implementation of the First Course of Pre-Medical Education of Uruguay. School of Medicine. University of Uruguay
- Member of the Commission for Reformulation of the Plan of Studies. School of Medicine. University of Uruguay
- Member of the University Cloister on behalf of the Faculty
- Member of the University Cloister on behalf of the Graduates
- Member of the Directive Commission of the Medical Residences Program (Coordination School of Medicine-Ministry of Public Health).
- President of the Directive Council of the Uruguayan Medical Corporation, MUCAM (Private Health System).
- Founder and Member of the Honorary Commission Against Cancer of Uruguay
- Founder and Member of the First National Bank of Antitumoral Drugs of Uruguay
- Founder of the National Breast Cancer Program of the Ministry of Public Health of Uruguay
- National Director of Health of the Ministry of Public Health of Uruguay

- Technical Director of the National Fund of Resources of Uruguay (National System of Financing Highly Specialized Medicine)

SCIENTIFIC SOCIETIES

- American Society of Clinical Oncology- ASCO – Active Member
- Society of Internal Medicine of Uruguay. Permanent member
- Society of Medical and Pediatric Oncology of Uruguay. Founder Member and First President
- Society of Hematology of Uruguay. Founder Member
- Society of Clinical Pathology of Uruguay. Permanent Member
- Argentina Medical Association. Founder Member (1982). Honorary Foreign Member (1982). Advisory Member (1987). Consultant Member (1987).
- European Organization for the Research of Cancer. Correspondent Member
- South American School of Oncology. Directive Member

SCIENTIFIC PUBLICATIONS-BOARD MEMBER

- Cancer. Spain
- Clinical Oncology. Association of Clinical Oncology, Argentina

SCIENTIFIC PUBLICATIONS-INVITATION AS REVIEWER FOR:

- AJCO. American Journal of Clinical Oncology, US
- APS. Acta Pharmacologica Sinica, China

THE MOST RELEVANT SCIENTIFIC PUBLICATIONS

Author or Co-author of 155 papers published in scientific journals and Medical Congresses Acts. Among those, 58 are publications in international forums, peer review journals. The following is a selection of the 10 most relevant publications related to the main field of research, maintained for more than 30 years.

- Extraction of an Inhibitor of DNA synthesis from human peripheral blood lymphocytes and bovine spleen. E. Lasalvia, E. García-Giralt and A. Macieira-Coelho. Rev. Europ. Etudes Clin. et Biol. 1970, XV: 789-792

- Evidence for to lymphocytic chalone. E. García-Giralt, E. Lasalvia, I. Florentin and G. Mathe. Rev. Europ. Etudes Clin. et Biol. 1970, XV: 1012-1015

- Suppression of Graft-vs. -Host Reaction by to Spleen Extract. E. García - Giralt, VH Morales, E. Lasalvia and G. Mathe. The Journal of Immunology, 1972,109,4:878-880

- Prevention of Graft versus Host Reaction by incubation of lymphoid cells with to splenic extract (Not Affecting the Repopulation of the Hemopoietic tissue). E. García-Giralt, VH Morales, B. Bizzini and E. Lasalvia. Cell Tissue Kinet, 1973,6:567-571

- Decreased lymphoid chalone rate in the spleens of lymphoma patients. E. Lasalvia, A. Luquetti, C. Oehninger, and E. García-Giralt. Proc. XV Congress of the International Society of Hematology, 1974

- Inducción autóloga de fibrogenesis tumoral (Autologous Induction of tumor fibrogenesis). Lasalvia E, Cucchi S, DeStefani E, Deneo H, Fierro L, Mechoso B, Larrañaga J, Vázquez J. Neoplasia (Spain), 1995(1): 5-10

- Anti-metastatic effect of a blood fraction from cancer patients. Lasalvia E, Cucchi S, Carlevaro T, Vázquez J, Riotorto R, Fierro L. Proc 31st Annual Meeting of ASCO, May 1995:Abs. 730

- Serum Markers Variation Consistent with Autoschizis Induced by Ascorbic Acid–Menadione in Patients with Prostate Cancer. Eduardo Lasalvia-Prisco, Silvia Cucchi, Jesús Vázquez, Eduardo Lasalvia-Galante, Wilson Golomar and William Gordon. Medical Oncology; 2003; 20(1): 45-52

- Antitumoral Effect of a Vaccination Procedure with an Autologous Hemoderivative. Eduardo Lasalvia-Prisco, Silvia Cucchi, Jesús Vázquez, Eduardo Lasalvia-Galante, Wilson Golomar and William Gordon; Cancer Biology & Therapy; 2003, 2-2:123-126

- Breast Cancer: Autologous Immunogenicity Elicited by Chemotherapy. Eduardo M Lasalvia, Silvia Cucchi, Jesus Vázquez, Eduardo Lasalvia-Galante, Wilson Golomar, William Gordon; Proc. 39th Annual Meeting of ASCO, May 2003: Abs. 746

- Insulin-induced Enhancement of Antitumoral Response to Methotrexate in Breast Cancer Patients. Eduardo M Lasalvia-Prisco, Silvia Cucchi, Jesús Vázquez, Eduardo Lasalvia-Galante, Wilson Golomar, William Gordon. Cancer Chemother and Pharmacol (2004) 53: 220-224.

- Colorectal Cancer: Autologous Immunogenicity in Chemotherapy (5FU) Pre-Treated Patients. E. Lasalvia-Prisco, S. Cucchi, J. Vazquez, E. Lasalvia-Galante, W. Golomar, F. Cotto, R. Otero; Proc 40th Annual Meeting of ASCO, May 2004, Abs. 2593

- Breast Cancer: Updated Vaccination With An Autologous Hemoderivative in Changing Tumor Antigen Library. E. Garcia-Giralt, E. Lasalvia-Prisco, S. Cucchi, J. Vazquez; Proc 40th Annual Meeting of ASCO, May 2004, Abs 2594

- Breast Cancer: Role of Tumor Associated Antigens and Regulatory Cells [CD4+CD25+] as Targets of the Immune Response elicited by an Anti-progressive Autologous Hemoderivative Vaccine. E. Garcia-Giralt, E. Lasalvia-Prisco, S. Cucchi, J. Vazquez, E. Lasalvia-Galante, W. Golomar. Proc 41ST Annual Meeting of ASCO, May 2005, Abs. 2589

- Colorectal Cancer: Comparative effects of an Autologous Hemoderivative Vaccine with a CEA vaccine and/or an autologous [CD4+CD25+] vaccine. E. Lasalvia-Prisco, E. Garcia-Giralt, S. Cucchi, J. Vazquez, E. Lasalvia-Galante, W. Golomar. Proc 41ST Annual Meeting of ASCO, May 2005, Abs. 2596

- Advanced Colon Cancer: Antiprogressive Immunotherapy Using an Autologous Hemoderivative. Eduardo Lasalvia-Prisco, Emilio Garcia-Giralt, Silvia Cucchi, Jesús Vázquez, Eduardo Lasalvia-Galante, Wilson Golomar. IN PRESS to be published in Medical Oncology, Medical Oncology, vol 23, no. 1, 91-104, 2006.

- Advanced Breast Cancer: Antiprogressive Immunotherapy Using a Thermostable Autologous Hemoderivative. Eduardo Lasalvia-Prisco, Emilio Garcia-Giralt, Silvia Cucchi, Jesús Vázquez, Eduardo Lasalvia-Galante, Wilson Golomar. Breast Cancer Research & Treatment. Printed: Online First: www.springer.com

- Prostate cancer: autologous immunotherapy optimized by indoleamine- 2,3-dioxygenase (IDO)-inhibitor as immune-tolerance breaker. E. Lasalvia-Prisco, E. Garcia-Giralt, S. Cucchi, J. Larrañaga. Proc. Am Soc Clin Oncol (ASCO), 42nd Annual Meeting 2006: Abs. 12509

- Ovarian cancer: autologous immunotherapy optimized by remote adjuvancy of a silicate -induced granuloma. E. Garcia-Giralt, E. Lasalvia- Prisco, S. Cucchi, E. Lasalvia-Galante, J. Vazquez, W. Golomar, J. P. Vincent. Proc. Am Soc Clin Oncol (ASCO), 42nd Annual Meeting 2006: Abs. 12515

LANGUAGES

English, Spanish, French

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Commentary

A New Twist on Autologous Cancer Vaccines

Leisha A. Emens

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Physicians have had a long-standing interest in marshalling the cancer patient's own immune system to effect tumor rejection. The use of cancer vaccines to activate an endogenous antitumor immune response has the advantages of exquisite tumor specificity, low toxicity, and potential durability due to the phenomenon of immunologic memory. Moreover, cancer vaccines are an attractive complement to the standard cancer treatment modalities of surgery, radiation therapy, and chemotherapy, offering a non-toxic treatment strategy that is likely to be non-cross resistant. Even with these advantages, the use of therapeutic cancer vaccines also poses significant challenges. Their efficacy is hampered by the extent of the tumor burden, relatively well entrenched mechanisms of tumor-specific immune tolerance, and the potential plasticity of the tumor cells themselves. From a practical point of view, the development of tumor vaccines is further limited by the technical limitations posed by the nature of the vaccination platform itself. In this issue of *Cancer Biology & Therapy*, Lasalvia-Prisco and colleagues report the results of the first clinical trial testing a novel vaccine formulation utilizing an autologous hemoderivative for the treatment of advanced solid malignancies.[1] While clearly preliminary, their approach is intriguing because it circumvents many of the practical obstacles to the development of effective vaccines for cancer therapy.

Tumor vaccine formulations can be broadly divided into those that are well defined, and those that are.[2] Well-defined tumor vaccines contain known tumor antigens; examples include peptide-based, protein-based, and plasmid DNA vaccines. These vaccines offer the advantages of relative ease of manufacture, clear targets for the monitoring of immune responses to vaccination, and a good safety record to date. One disadvantage to the use of precisely targeted tumor vaccines is that many tumor antigens seem to activate antigen-specific immunity that is incapable of mediating a tumor rejection response. This concept is supported by the results of studies characterizing the natural and vaccine-induced immune responses in melanoma patients who continue to have disease progression despite the presence of antigen-specific tumor immunity.[2-7] A second disadvantage to highly targeted cancer immunotherapies is that they favor the selection of antigen loss variants, ultimately resulting in the outgrowth of a subpopulation of antigen-negative tumor cells that are by definition resistant to therapy.[8-10]

Tumor vaccines that are less well defined are generally formulated either directly from tumor cells themselves to make a cellular vaccine, or are derived from tumor cells as a crude preparation of viral lysate or heat shock protein (HSP)-peptide complexes.[2] There are two primary advantages to cancer vaccine formulations that are less well defined. First, these vaccine platforms deliver a variety of tumor antigens. By definition, they are capable of directing the immune response simultaneously to multiple antigens, greatly decreasing the probability of immune-mediated selection of antigen loss variants. Second, the menu of antigens delivered can include both known and as yet unknown tumor antigens. The sheer number of antigens delivered thus increases the likelihood of activating the immune system to recognize an antigen that can mediate tumor rejection. One disadvantage to the use of undefined antigen vaccines relates to the primary antigen source. The use of autolgous tumor cells is often preferred due to the possiblity that critical targets for immune-mediated tumor rejection are unique to each tumor. However, sufficient numbers of autologous tumor cells are frequently not available to support full vaccination regimens, leading some to investigate the use of allogeneic tumor cells to deliver tumor antigens common to a given histology. A second disadvantage of using relatively undefined vaccine platforms is that there is often no clear target for monitoring vaccine-induced immmune responses. Some investigators have used the development of delayed type hypersensitivity (DTH) to autologous tumor as an informative measure of vaccine-induced antitumor immunity.[11-22] Again, this is possible only when autologous tumor is available for processing, and this is frequently not the case for advanced solid tumors. A third drawback to less defined vaccine formulations is that the quality of the manufacturing process may be difficult to ensure.

In particular, the antigenic content of autologous tumor cell-based formulations will be unique. Appropriate measures of manufacturing consistency and potency may thus be more difficult to define for cell-based as opposed to highly targeted cancer vaccines.

Lasalvia-Prisco and colleagues describe a novel vaccine formulation derived from the arterial blood of advanced solid tumor patients. They develop a procedure for manufacturing and partially characterizing an autologous hemoderivative, and then test it as a cancer vaccine in a clinical trial involving patients with a variety of advanced solid tumors. The processing of the vaccine itself is simple. It is derived from 20 milliliters of femoral arterial blood. After sedimentation at 37°C, the supernatant of plasma and cells is subjected to hypotonic shock, followed by freezing. Twenty-four hours later the preparation is thawed, exposed to 100°C for 10 minutes, and filtered over cellulose acetate. A crude analysis of the vaccine preparation showed it to consist of a minimum of five protein fractions, with a major homogeneous protein component of approximately 50,000 kD. Although they were present prior to processing, heat shock proteins and known tumor markers were not detected in the final product.

Although it remains relatively uncharacterized, this vaccine platform in principle offers multiple advantages. First, the small quantity of arterial blood required is quickly and safely accessible by femoral arterial puncture. Second, the manufacturing process is relatively simple and cost-effective, requiring minimal manipulation and no direct chemical or genetic modification of the cellular component prior to processing. Finally, and most importantly, the patient himself is a renewable source of therapeutic material. At the time of tumor recurrence, an updated vaccine product that accurately reflects that antigenic profile of the tumor at that point in time could be easily obtained and prepared.

The investigators designed the clinical trial to overcome some other limitations to the efficacy of therapeutic cancer vaccines. They include GM-CSF, a cytokine that is well known for its immune-stimulating properties, as a local vaccine adjuvant. They also incorporate a low dose of intravenous Cyclophosphamide three days prior to vaccination. Although chemotherapeutic immunomodulation is not widely used in cancer vaccine trials, there is substantial preclinical and clinical evidence to suggest that Cyclophosphamide can augment the induction of antigen-specific immunity.[18,23-26] Importantly, the trial also included a control group that received Cyclophosphamide and GM-CSF but no autologous hemoderivative. The vaccinated group had a higher frequency of stable (SD) or responding (PR) disease than did the control group ($p < 0.001$). Also, the vaccinated patients demonstrated a correlation between clinical response (SD + PR) and the development of DTH to the autologous hemoderivative of at least 5 mm in diameter ($p < 0.02$). Moreover, histologic analysis of responding metastatic lesions were characterized by stromal fibrosis and CD^{3+} T cell infiltration not characteristic of pre-treatment biopsies.

The mechanism underlying the reported bioactivity of this treatment approach remains an open question. Although no HSPs are found in the final vaccine product, the overall vaccine preparation is reminiscent of HSP cancer vaccines.[27] It is possible that a stress-related protein present in the vaccine preparation delivers tumor-derived antigens present in the arterial blood in a form capable of activating T cell-dependent immunity. This is supported by the development of DTH to the hemoderivative, and by the presence of T cells infiltrating the responding tumors. It would be more strongly supported by additional measures of antitumor immunity, such as DTH to autologous tumor where available (rather than the autologous hemoderivative), or cellular immune responses to a defined antigen known to be present in the patients tumor (by ELISPOT).

Interestingly, the manufacturing process includes a step of hypo-osmotic shock. Hypo-osmotic shock is known to activate monocytes and macrophages, enhancing phagocytosis and the secretion of cytokines such as interleukin 1 and interleukin 6.[28,29] The contribution of this step to the bioactivity of the vaccine is unclear, but it could provide a vaccine adjuvant that is an integral component of the vaccine itself.

The most striking histopathologic feature of responding lesions is the presence of marked fibrosis. The extent of this fibrotic response suggests an additional mechanism distinct from that mediated by T cell against the tumor cells themselves. The observed histology strongly argues for a therapeutic effect that shifts the balance of interactions between the tumor cells and the supporting stroma to favor tumor regression. Carefully elucidating the regulatory pathways underlying this aspect of the hemoderivative's bioactivity should facilitate the development of informative surrogate measures of clinical response for use in future clinical trials.

In summary, Lasalvia-Prisco and colleagues have described a novel cancer vaccine platform consisting of an autologous hemoderivative, with a suggestion of clinical response. These results are preliminary, and require confirmation in larger trials and by other investigators. Further characterization of the critical parameters of vaccine formulation and the mechanism of bioactivity will facilitate the development of more informative clinical trials. If these results are confirmed and extended, this vaccine platform represents an exciting development in the field of cancer immunotherapy. The ability to re-derive a potent vaccine in response to the changing antigenic profile of an evolving metastatic tumor is a powerful and unique feature of this vaccine platform. Clearly, an active, individualized cancer vaccine that is cost-effective and simple to manufacture would be a welcome addition to the treatment armamentarium for metastatic solid tumors.

References

1. Lasalvia-Prisco E, Cucchi S, Vazques J, et al. Antitumoral effect of a vaccination procedure with an autologous hemoderivative. Cancer Biol Ther 2003; 2: In press.
2. Emens LA, and Jaffee EM. Cancer Vaccines: An Old Idea Comes of Age. Cancer Biol Ther 2003; 2 (Suppl 1): In press.
3. Romero P, Dunbar PR, Valmori D, et al. Ex Vivo Staining of Metastatic Lymph Nodes by Class I Major Histocompatibility Complex Tetramers Reveals High Numbers of Antigen-Experienced Tumor-Specific Lymphocytes. J Exp Med, 1998; 188:347-53.
4. Anichini A, Moll A, Mortarini R, et al. An Expanded Peripheral T Cell Population to a Cytotoxic T Lymphocyte (CTL)-defined, Melanocyte-Specific Antigen in Metastatic Melanoma Patients Impacts on Generation of Peptide-Specific CTLs But Does Not Overcome Tumor Escape from Immune Surveillance in Metastatic Lesions. J Exp Med. 1999; 190:651-7.
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